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Liew, Choong-Chin

<120> Method for the Detection of Gene Transcripts in Blood and Uses  
Thereof

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<150> US

<151> 2000-01-04

<150> US 60/115,125

<151> 1999-01-06

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<170> PatentIn version 3.2

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 45 caaataaaca aaaacttcca atctccattt aaaat 155

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 35 ggaacaaatt tgggataaaa atataaggct gggattacag gcatgagcca ccaagcccgg 120  
  
 cccacatttc cattttaat atatactgtg ctttacaat attataatat gttttaaatt 180  
  
 40 atgttcacag aagcacctgg tctgtgaatg gcatgccagc attaaaaaaaa ataagcattc 240  
  
 tttgaatata tatttagttt ttaattgtg taggaaaatc aaagccagag ggagtagaaa 300  
  
 caaaatttgt gattttctaa atacttctg gctgcaggga agaaaccacg tcccaggcga 360  
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438

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 15 aaagggggaa ggcccccaa accggggggg gggnaaaaag gtgggtttcc cccttttcc 180  
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 45 caaattgggg ggnaaaccaa atttaagggg ggaagggggg gnccccccg ggaaaggccc 180



aaggggggaa aatttttccg ggggtgggtn gggggaacca atttaagggg ggggcccccg 240

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15  
 ctgtacttga actctaaaac tgttgagaaa actcagtgtt tacccaaca gattcatttc 180

aaatagctgt aaaaggtatg ttactccag aagaccagag ttgtctttt tgaacttctc 240

20  
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ccataaacag cactccctca ggcccctgcc tgacacaggc atagactgtc atgttgatt 360

cacagacagg ctgtgctaga ggaaacctct ggggctcacc aggggccgtg ggatgggctt 420

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 tttaggcggc accccaacaa caccaggccc tactttttcc aaggncgggg aagcccatgg 180  
 45 gttctgggna acgggcaatg cgggcttgca acgggnggaa naaaaacagn cccaaaagaa 240

tg 242

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 tctcagctca ctgcaacctc cacctctcag gagaattgct gaacctggga ggcggaggtt 120  
 15 gcagggagct gagattgcgc cactgccctc catcctgggc gacagagcaa gaacctgtct 180

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 cggaaggcc nttggggaaa aaaccaangg ncaaaccccc ccaaccacnc ggcccccccc 120  
 15 aaggggggtg gggaagagcc aaatttcttt gggaanaac gccccttgg ggaaaanaag 180  
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 30 ggaagaattg tatgtgggag ttttaatgg tctcatttca ttggctataa ctcaattaca 180  
 aggagaaata taactgcaga ggagcttga aaatttagtt cagctgaggg taaaggaaga 240  
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40 ttgaggcgaa aggactcgcg gatttgacgc ttgatgcggt tgcgctcgac ggcgagcttg 180  
  
acgctctttt tgccgatcac caaacctagg cggggatgat caagctgggt atcgcgcgct 240  
  
agcagcagga cacttttgcc cgggagcttt accgcttggg gagtcgaaga ctgccttгна 300  
45 ttgccgggga gtcagcagtc gctttttccc ggncgaagcc tcgaactcac cancctgtct 360

ggattaatta gacagcaaga cgcttgcggc ccctttggcg cgaacgaacn ncgaaaagga 420

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acgagccggg ctggagcggg gggaaaagca aatgtaggca taagtaacga caatgcgggc 120

45 gagaaccccg cacaccgaaa ggctaaggat tcctccgcta tgtcaatcaa cggagggtta 180

gtcgggtact aaggcgtag cgaaggcgaa gcgccgatgt gaaggggggt aatattctc 240  
cacttgccat gcgtgtgaat ccatgacgga gacgaagccg ggggtgcgtc ctgacggaag 300  
5 tgggcgccag caggggcggc cttcgggcca aaccgaacct caggtcanac ttccaagaaa 360  
agtgggtgaa acgccagcgc atggcaaccc gtaccgcaaa ccgacacagg tagccggggg 420  
anaacatcct aaggngctcg agagtacttt ctagagcggc cgcgggcccc atcgantttt 480  
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gctggaaggc ttgnagggca gaaaagggat ccgcctttgg gaggaacca ggtaaggttt 120  
  
aagaaggaac ccacctngg ggccaaacaa aaacttaaaa acccccccat ttentncccc 180  
20 ccaaaaaaaaa aatttttaaa aaaaattttt ngcccccggg ggcattgggg g 231

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attcggcacg agggattaca gtcgtgagcc actgcacctg gctgcaatt 109

10 <210> 30

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30 ggtgctcctg cgtttgact gtgcaggcct ctctatgct gatgcgtct cccactcctg 180  
  
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 35 actaatgaaa tgaaaatgaa atgganntga tgagatgaga agaantgctg agatgagata 420  
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cantnnnctg gggncaccn nnnngaact gagagtggat ctgtgtacc tgacnnacca 240

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ccngnctatc tcaactganga tgattgncag ngccnntggg tggcacnang ggnactgatg 360

25 ancancactg accctgccga cgccagangc cgcanatccg gagantncat gngacnata 420

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gaagagtctt agttcattta ttggcccag aaccctctaa tccccttgta attatgtca 240

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aataatcagg caaatcctta agaataaggg caattaagga tgactagccc tacaagattt 180

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taaaaaggat tcattagttt aaaaaatgtg atgtagatac atgaataaaa taaaatcttg 240

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atgaagtga ataataaaaa tatattctac actagcacia attataaata aagcagtgat 420

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agatgaatga tgaaatgaaa tgatgagatg agatgatgaa atgaaatggt gagatgaaat 240

gatgagatga aatgaaatag tgaaatgaaa ttgaaataaa atcgaaatga gagatgaaat 300

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 15 ngacgcgccg tacaggntna attatgggnan cttacatggn aaaggggcan ctcaatgtcc 120  
  
 cacag 125  
  
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 35 aaatgaatct gagatgaaat gagatgaaaa ntgatacgaa aaatgatata aaaaatatga 180  
 cctgagatga aatgagatga aaaatgatac gaaaaatgat ataaaaaata tgacatgaaa 240  
 40 tgaaatgaga tgatatgaaa tgacataatg aaatgatgaa ttgatgatat tgaaatgaaa 300  
 ttgaaagatg agatgaaatg atgagatgaa atgaaatgtt gaaatgatga agagatgtga 360  
 catgaaatga gctgaaatga gatgaaatga aatgagatta aatgatgaga tgaaaaatga 420  
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 gaaatgagat gaaataaaat aatgaaatga tgaaataatg aatgaaaat gagatggaaa 180  
 30 tgatgagatg agaagaaatg atgagatgaa atgatgaaat gatgagatga ganaaaatga 240  
 gatgaaatga tgagatgaga tgaaatatga tgagttgaaa tgacataatg aatgaaatga 300  
 35 tgaaatggaa taatgaaatg gaaatgatga gctgagatgc aatgagttga aatgagatga 360  
 aatgatgaaa tgatgagatg aatgatgaa atgaaataat gaaatgagat gaaataaaat 420  
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<223> n is a, c, g, or t

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cataaaatga gatgaaatga gatgtaatga tggaatgaga tgagatgaaa tgagatgaaa 180

20 tgatagatga gataaaatga tgatatgaaa tgatgagatg aatgatgaga tgatgagatg 240

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<210> 75

<211> 155

<212> DNA

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ctactnaga ngaantcctc gcaaaatcct tgaatcaccg cttcagtga gtgatcac 120

cgccatgaaa ttctgctcg attagcttac gttgttgga tagaggcaa acaaggctgt 180

45 tatcggtagc aggaatggat gttcgatttc gtagaatacg cctgagagac ggccaatact 240

ctcacgagag gcagcaggcg cgtaaattac ccaattacaa caagtagagg tagcgaagga 300

aaatatgagg ggtggcaagg tttgcctgt tacattctca aatggaagca aattagatat 360

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gtcattg 367

<210> 77

10 <211> 257

<212> DNA

<213> Human

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atagaaatca ttatttctgt gggatggata attgggccaa attgtaaaat attttaacta 120

30 tcagtgtttg gggtttattt taaaagaat aggggtccac cagatgttct ttagtggagg 180

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aggtcctttc ttagtct 257

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<211> 373

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 30 tattacaaa atattggac cattantaaa gantagggcc aaccnaatt ttcttgaaa 180  
 ttccgttaa atagccgta aatgtttta cccattcat attggatacc ttaaattata 240  
 35 ataatggatt ttattgttaa attgtgtgtg tgtggtgtgt atgccctgtc ttctctctc 300  
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<400> 79  
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35 cccttgatggtt caagtattta agaccctatg cnaatatttt ttaccttttc taataaacca 120

tgtttggtt 128

40 <210> 80  
 <211> 213  
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ggccaggcct tcctttaaa aaaaaatnac cgttttngg gggangnaac ctttaaaagg 120  
  
ggaaaanaaa tcctttttaa anggaantcc aagggaagga ncctgnncaa nacttcccn 180  
30 ccaataaaaa aaacnntttt ggaaangggg aaa 213

<210> 81

35 <211> 443  
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<223> n is a, c, g, or t

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<223> n is a, c, g, or t

<400> 81

10 gaaatgagat gaaacatga gnatgaaatg aannaatgnc atgcaaatga tgagatgaaa 60

tgatgaaatg agatgagatg agaagaaatg acttgatgag atgagataaa atgatgaaat 120

gaaatgaagt gaaatgaaat tgaaatgaga tgagatgaaa tgagataaaa tgatgagatg 180

15 aaatgagaag aaatgagatg aaatgatgaa atgatgagat gagatgaaaa atgatgggat 240

gagaaatgag atgaaatgat gggatgaaat gaaatgaaat aatgaaataa tgaaatgaaa 300

20 tgaattgata atattgaagt gaaattgaaa gatgagattg gatgaaatga tgagatgaaa 360

tgaaatgttg aaatgaaatg aagagatgta acatgaaatg agctgaaatg atgagatgaa 420

atgaaatgaa atgagattaa atg 443

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<210> 82

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 15 <223> n is a, c, g, or t  
  
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 25 gacctctgaa tgtattantg anncaagtga tagccaagat attttgaaga aaaatagata 120  
 ntagggacct gctctataag cccatcataa ttattatga agttataaca agtaaaacag 180  
 taaggtattt ggcatggaat agagaaccca gaaacagacc caatgcatgg gtacaggata 240  
 30 taacacaggg aaatgaggga caatatatgg ttctgggata attatttata tggggaaaat 300  
 aaagaaattg gatccctacc tcacacatac aaaaaaaatc ataattgaat taaaaacttg 360  
 35 catgtgaaag gaaagacttt aaaacattta gaaaaagtat tggaggctat gatcttgggg 420  
 taggaaagca ttctttttt tt 442  
  
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 aggagggaaa agaaagaaag gaaggaagga aggaaaagaa agaaagaaag gaaggaagga 120

15 aggaaggaag gaagg 135

<210> 84  
 <211> 346  
 20 <212> DNA  
 <213> Human

<220>  
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 <223> n is a, c, g, or t

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 aacttctca gaaggacaag agacaaagaa gtgggggagg ccctcctatc catagctgag 180  
 35 agggtttatt ctttgtggtt ctgctgtcag agcctttgga tgtctgatct gagatggagc 240  
  
 aaccccgact agacagaact ttgtagattt tgggggggtt aaaaggcctc aagcaaattc 300

40 taaaactttc ttgaacccc ctggcatagg ctgagtttcc ctgact 346

<210> 85  
 <211> 100  
 45 <212> DNA  
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<400> 85

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5 gaaaagaaaa tggtttttt tgccgaaatc aaccgggtaa 100

<210> 86

<211> 201

10 <212> DNA

<213> Human

<400> 86

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15 gaaaagagga agaaacgctt ttaggaggtt aaggattaaa gtaaaaatta ttttgacata 120

attaccttct tttgtgacca ctcttaaagg ccaggaacat atttgagagaa gcctagtgtg 180

20 atgtaacagt gtgggggttc a 201

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25 <212> DNA

<213> Human

<400> 87

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tcttttacc cggcactcgg gtccaccctc gcggcaccag aggtattctc cggcgagtcg 180

35 ttaaccatcg caatcgccga ccgagtttaa ggaccactcc ccacctttct cattagttaa 240

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gtcgagtccc tcttctctc tttatgcatt tagagcgctt tcgtaagagt tttccctaga 360

40 ttttctaag cgtagcgcgt ctactccaat gtttcgtta atccagccc aactaacgcc 420

gcggaggagt cgatccgtct actcctatcc cgtcggctcg gatttactac aggagctaag 480

45 aaaacaaaa gtaccagccc taaaggaaag tcaaaggacg cccgtaaaaa a 531

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 5 <213> Human  
  
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 acagccaaac cggtcgcccc aaggcctccg tcgttttata atatattccg ttacgtata 180  
 aggaacgaac ccccttcat taccacggtc ccgcgtccgc ctcttctcc attcgcaaca 240  
 15 gttctattcc ttacagctc ccgtacctgc ttccagaaca tcgcaccgcc atagtcgaaa 300  
 gatagcaaag attaccagc ttctattcct cgccccagag ccgagtaaatt cgaagtttat 360  
 20 agaggcggaa tccaaccatt caagagttat aacaagttat cggcactcgg gggatcagaa 420  
 tataaactta atgtccctt tattctcccg gacgcccctt ttaaccactt ctctctatct 480  
 ttcgtaaca agccattgac ggcgctttgc cgcgcggggc catctcgcgt 530  
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 30 <213> Human  
  
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 aattaaggna aggnntaaaa aatttaacca anggggggtt taaagggnnt tttttttta 180  
 aaaaaaaagg taaancccc ccctttttt ttgggttggg gtgggaaaat tttgggaanc 240  
 45 cttaaccccc gggtttttgg gttttttgg ccaaaccgcc cgggaaaaaa attaaaaaaa 300



ggaccggttt ccattttaat gggattggg aa 332

5 <210> 90  
<211> 185  
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<213> Human

10 <400> 90  
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cggtaggaac tattaaaaca taagttaaat cggattttaa aattttaaaa tcaaaaaaaaa 120  
  
15 taaaatatat gcaaccctcc attcaagga agtatgatgt tactagatta tctgaaaatt 180  
  
ctcct 185

20 <210> 91  
<211> 365  
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<213> Human

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10 ntaaatgnga tgaattaatg atgagatgaa atgntgaatg ccgggggtgnn tgagatgaat 720  
tgagtgaac cctgngatga atgaagattg nntgaatggt ggntgaatgt tgaatggntg 780  
15 gntggnanaa tgcctgtngg 800

<210> 97  
<211> 334  
20 <212> DNA  
<213> Human

<400> 97  
gatgaattga aatgaaatga aataatgaaa taatgaaatg agatgaaatg aaaagaaatg 60  
25 atgaaatgat attgaaatga aattgaaaga tgagatgatg agatgaaatg gtgaaatgtt 120  
gaaatgaaat gatgaaatga atagatgtga catgaaatga gctgaaatga tgagatcaaa 180  
30 tgaaatgaaa tgagattaaa tgatgagatg aaaactgatg aaaacttaaa tgatgaaata 240  
atgaaatgaa aatgaaatgg aaatgatgag atgagaagaa atgatgagat gagatgagat 300  
aaaatgagat gaaatgatga gatgaaatga tgag 334  
35

<210> 98  
<211> 100  
<212> DNA  
40 <213> Human

<220>  
<221> misc\_feature  
45 <222> (17)..(17)  
<223> n is a, c, g, or t

<400> 98

ttcaggccgt ctgctntac atatactatc gagaatggtg ctgtgcactc ataacaccgt 60

5 tgcttggttag acgcttttga acccttcagc gctgaaagta 100

<210> 99

<211> 500

10 <212> DNA

<213> Human

<220>

15 <221> misc\_feature

<222> (8)..(8)

<223> n is a, c, g, or t

<220>

20 <221> misc\_feature

<222> (361)..(361)

<223> n is a, c, g, or t

<400> 99

25 cccggganntt cggcccttat ggcccgggga aatgatgaga tgaaatgatg aatgagata 60

agatgaaaag agttgatgag atgatgagat gaaatgagat gaaaagagat gaaatgagat 120

gaaatgaaat gatgagatga aatgaggtga aatgaaatta gatgaaacgt aatgagatga 180

30 aatgacctaa tgaaatgaaa aatgaaatg aaataatgaa atgaggtgaa attaaatgag 240

atgatgaaat taaatgatga aatgaaataa tgaaatggaa atgaaatgga aatgatgaga 300

35 tgaatgatga gatgaaatga tgagatgaga tctaatgatg agaggagatg atgagatgaa 360

ntgagatgaa aagagatgaa atgagatgaa accgaaatga tgagatgaaa tgaggtgaaa 420

tgaaattaga tgaacgtaa tgagatgaaa tgacataatg aaatgaaaaa atgaaatgaa 480

40 ataatgaaat gaggtgaaat 500

<210> 100

45 <211> 397

<212> DNA

<213> Human

<220>

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<220>

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<223> n is a, c, g, or t

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15 <221> misc\_feature  
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<220>

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<223> n is a, c, g, or t

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25 <221> misc\_feature  
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<220>

40 <221> misc\_feature  
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<223> n is a, c, g, or t

<400> 100

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tccccttgaa nntctctaaa ccttaacttt ctggccnttt tgtttcagan atgcctaacc 120  
 ctcagggggt cttttgttct ctacgcctaa aaacttaatc tgtttggaac aattccnttt 180  
 5 cctctctgta gaaattgacc tggccatggc tcctgtgaat gatacgggtg ctattatccc 240  
 tgaacactgt aaaaatgaac ttgaaacag ttgggttagga cccaaacaga aaatgatgta 300  
 tggcttgga atagtttagc tgaacattat gctttaatat ttactggcc attgcagcac 360  
 10 aggtttagaa atttatgttc ggcttttta agtttta 397

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 15 <211> 132  
 <212> DNA  
 <213> Human

20 <220>  
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 <223> n is a, c, g, or t

25 <400> 101  
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 attaatgta ctactttcaa aagaattaat tgaagaaaga gagatacagg gtatctaggc 120  
 30 ngaggaagac cc 132

<210> 102  
 <211> 246  
 35 <212> DNA  
 <213> Human

<400> 102  
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 40 ggcactta atttataattg atatatataa taaaaaatc aatgcagata ttgatataat 120  
 aaaaatagat aatggtaatc caagcacgat ggtagccatc actctaattg ctttgggggtt 180  
 45 aacctataac ttattaagta aagtccaga atgggtcttt gacagtatta aaattaaaga 240



aaacag

246

&lt;210&gt; 103

5 &lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

10 <223> forward primer of exon 1 of insulin gene used for quantitative  
RT-PCR analysis

&lt;220&gt;

15 &lt;221&gt; primer\_bind

&lt;222&gt; (1)..(18)

&lt;400&gt; 103

20 gccctctggg gacctgac

18

&lt;210&gt; 104

&lt;211&gt; 18

&lt;212&gt; DNA

25 &lt;213&gt; Artificial Sequence

&lt;220&gt;

30 <223> reverse primer of exons 1 and 2 of insulin gene used for  
quantitative RT-PCR analysis

&lt;220&gt;

35 &lt;221&gt; primer\_bind

&lt;222&gt; (1)..(18)

&lt;400&gt; 104

cccacctgca ggctctct

18

40 &lt;210&gt; 105

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

45 &lt;220&gt;

<223> forward primer of  $\beta$ MyHC gene used for quantitative RT-PCR

analysis

<220>

5 <221> primer\_bind

<222> (1)..(24)

<400> 105

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24

10

<210> 106

<211> 24

<212> DNA

15 <213> Artificial Sequence

<220>

<223> reverse primer of  $\beta$ MyHC gene used for quantitative RT-PCR  
analysis

20

<220>

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<222> (1)..(24)

25

<400> 106

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24

30 <210> 107

<211> 20

<212> DNA

<213> Artificial Sequence

35 <220>

<223> forward primer of ANF used for quantitative RT-PCR analysis

<220>

40 <221> primer\_bind

<222> (1)..(20)

<400> 107

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20

45

<210> 108  
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 <212> DNA  
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5

<220>  
 <223> reverse primer of ANF used for quantitative RT-PCR analysis

10

<220>  
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<400> 108

15

gcagatcgat cagaggagtc

20

<210> 109  
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 <212> DNA  
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20

<220>  
 <223> forward primer of APP used for quantitative RT-PCR analysis

25

<220>  
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 <222> (1)..(20)

30

<400> 109  
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20

<210> 110  
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 <212> DNA  
 <213> Artificial Sequence

35

<220>  
 <223> reverse primer of APP used for quantitative RT-PCR analysis

40

<220>  
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45

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19

5

<210> 111  
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<212> DNA  
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10

<220>  
<223> forward primer of ZFP used for quantitative RT-PCR analysis

15

<220>  
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20

<400> 111  
cacargagrc arggtcaacg a

21

25

<210> 112  
<211> 22  
<212> DNA  
<213> Artificial Sequence

30

<220>  
<223> reverse primer of ZFP used for quantitative RT-PCR analysis

35

<220>  
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ggattaaaat gaagcaccca ga

22